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Malt-Yeast Extract-Sucrose Agar, a Suitable Medium for Enumeration and Isolation of Fungi from Silage

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A general medium named malt-yeast extract-sucrose agar (MYSA) containing oxgall was designed. The medium was intended for the enumeration and isolation of molds and yeasts in routine examinations of animal feed stuffs. In this study MYSA was tested as a general medium for mycological examination of silage. The medium was compared with dichloran-rose bengal medium (DRBC) in an examination of more than 500 specimens of big bale grass silage. Selected characteristics of known fungal species commonly isolated from feeds were examined after growth on MYSA and DRBC and on malt extract agar, used as a noninhibitory control medium. MYSA suppressed bacterial growth, without affecting the growth of fungi common in feeds. The fungi growing on MYSA were easily recognized, and the medium seemed to slow radial growth of fungal colonies, which permitted easy counting. The number of species found was higher on MYSA than on DRBC. When we compared MYSA with DRBC for mycological examination of grass silage samples, MYSA was found to be the medium of choice.

The media for the isolation and enumeration of molds and yeasts from foods and feeds have been through a refining process. The general opinion used to be that a single all-purpose medium was sufficient for any commodity. Clearly, it is not so. Different fungi have different requirements, and the ideal enumeration medium should fulfill the following eight criteria (3, 25): (i) it should suppress bacterial growth completely, without affecting growth of food fungi; (ii) it should be nutritionally adequate and support the growth of relatively fastidious fungi; (iii) it should slow the radial growth of fungal colonies to permit counting of a useful range of numbers per plate, without inhibiting spore germination; (iv) it should suppress, but not totally prevent, the growth of spreading molds, so that they can also be enumerated; (v) it should promote the growth of fungi contaminating or spoiling the commodity examined; (vi) it should suppress the growth of irrelevant fungi; (vii) it should be easy to prepare; and (viii) the developing fungi should be recognizable without subcultivation.

At the Central Laboratory, State Veterinary Laboratories of Norway, a medium was developed on the basis of the criteria listed above and was intended for use in routine examinations of animal feeds. The main reasons for doing so were to reduce the number of media used for standard examinations and to reduce the need for subcultivation for identification. The aim was to produce a medium that would easily differentiate among various species of field fungi and storage fungi in grains and compound feed. Malt-yeast extract-sucrose agar (MYSA) contains some of the nutrients found in both malt extract agar (MEA) and Czapek yeast extract agar (CYA) (24). Oxgall was included in the medium to reduce radial growth without suppressing sporulation. The medium, named MYSA, has been used as the standard medium for routine mycological examinations of animal feeds in Norway for several years (31).

The aim of this study was to obtain information about MYSA as a medium for routine mycological examinations of silage. In several studies, dichloran-rose bengal medium (DRBC) (18) has been compared with a number of other media used for the routine enumeration of molds in foods and feeds and attained high scores (3, 7, 15, 17, 19, 27, 30, 32). In the present study, MYSA was compared with DRBC in mycological examination of more than 500 specimens of big bale grass silage. Selected characteristics of fungal cultures of current interest were also examined after growth on MYSA and DRBC. MEA was used as a noninhibitory control medium.

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MATERIALS AND METHODS

Samples. Altogether, 201 different strains of 20 fungal species commonly isolated from feeds and 571 big bale grass silage samples were examined. The silage samples were collected in different stages of two storing seasons and from all over Norway.

(MYSA). MYSA consisted of malt extract (Moss Aktiebryggeri A/S; 15 g), tryptone (Bacto; 2 g), oxgall (Bacto; 2 g), sucrose, (30 g), yeast extract (Difco; 5 g), sodium nitrate (0.5 g), agar (Sorbigel; 20 g), and distilled water (1,000 ml). The final pH should be 6.8 ± 0.2, after adjustment with 1 M NaOH. After sterilization and cooling to 45°C, 10 ml of 0.5% chloramphenicol (Sigma) and 10 ml of 0.5% chlortetracycline (Sigma) were added.

DRBC. DRBC was prepared according to the original formula (18), but chloramphenicol was added at the concentration recommended by Hocking (16).

Examination of characteristics of current fungal cultures. The fungal species and number of strains examined are listed in Table 1. Authentic cultures of the following species of fungi were purchased from the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands; the Fusarium Research Center (FRC), Pennsylvania State University, University Park; the Institute of Biotechnology (IBT), Technical University, Lyngby, Denmark; and the Centre for Agriculture and Biosciences International Mycological Institute (IMI), Egham, United Kingdom. The following strains were used: *Alternaria alternata* (CBS 154.31), *Aspergillus candidus* (CBS 566.65), *Aspergillus flavus* (CBS 573.65), *Aspergillus fumigatus* (CBS 110.46), *Cladosporium cladosporoides* (CBS 169.54), *Eurotium herbariorum* (CBS 516.65), *Fusarium avenaceum* (FRC R-4954), *Fusarium culmorum* (FRC R-8545), *Fusarium poae* (FRC T-532), *Mucor circinelloides* (CBS 203.38), *Mucor hiemalis* (CBS 242.35), *Penicillium citrinum* (CBS 139.45), *Penicillium expansum* (IBT 6102), *Penicillium fellutanum* (IMI 140 344), *Penicillium roquefortii* subsp. *roquefortii* (IBT 10101), *Penicillium roquefortii* subsp. *canescens* (IBT 6884), *Penicillium verrucosum* (CBS 603.74), *Rhizopus stolonifer* (CBS 112.07), and *Trichoderma viride* (CBS 256.62). Isolates of *Microdochium nivale* were kindly given to us by Anne Marthe Tronsmo, Norwegian Plant Protection Institute, Ås. The remaining cultures examined were isolates from feeds from the strain collection of the State Veterinary Laboratories of Norway.

Dishes were inoculated at three points per 90-mm-diameter plate of MYSA, DRBC, and MEA. All plates were incubated in the dark in plastic bags at 25°C in normal atmosphere and examined after 7 days. The characteristics observed were colony size and density, production of aerial mycelia and conidia, and

TABLE 1. Absolute minimum, maximum, and mean colony sizes of 7-day-old cultures growing on MYSA, DRBC, and MEA and colony sizes of cultures growing on MYSA and DRBC relative to colony sizes growing on MEA

Species	No. of isolates	Colony size (diam, mm) on medium[a]:									Colony size on indicated medium relative to that on MEA (%)	
		MYSA			DRBC			MEA				
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	MYSA	DRBC
Alternaria alternata	5	29	43	37	18	35	24	33	60	50	74	48
Aspergillus candidus	9	16	22	19	4	17	11	15	22	18	106	61
Aspergillus flavus	5	40	60	54	23	40	31	57	60	59	92	53
Aspergillus fumigatus	9	35	50	41	11	40	27	50	70	62	66	44
Cladosporium cladosporoides	16	5	18	11	3	12	7	6	17	12	92	58
Eurotium herbariorum	11	7	15	13	4	12	9	8	22	18	72	50
Fusarium avenaceum	16	40	65	49	7	42	16	55	70	65	75	25
Fusarium culmorum	16	60	70	69	12	55	22	70	70	70	99	31
Fusarium poae	16	20	70	51	7	45	20	45	70	64	80	31
Microdochium nivale	10	17	37	28	2	33	10	22	75	55	51	18
Mucor circinelloides	8	65	70	69	13	45	23	65	70	69	100	33
Mucor hiemalis	1	70	70	70	18	18	18	70	70	70	100	26
Penicillium citrinum	4	20	23	21	11	16	13	16	25	20	105	65
Penicillium expansum	16	20	32	27	13	24	20	25	45	35	77	57
Penicillium fellutanum	3	8	12	10	5	7	7	5	13	10	100	70
Penicillium roquefortii I	16	35	60	50	40	60	51	45	60	56	89	91
Penicillium roquefortii II	16	40	52	48	35	60	49	45	60	53	91	92
Penicillium verrucosum	10	10	30	19	8	22	14	11	45	21	90	67
Rhizopus stolonifer	6	60	70	68	55	70	68	60	70	68	100	100
Trichoderma viride	8	30	70	65	22	41	32	65	70	69	94	46
Total											89	57

[a] Min and max, minimum and maximum colony sizes, respectively.

surface and reverse colony pigmentation. Apart from colony size, colony characteristics were evaluated subjectively. The production of aerial mycelia and conidia was characterized as not visible (−), poor (+), average (++), and abundant (+++). Colony density was recorded as low (+), average (++), and high (+++). Colony appearance on MYSA and DRBC in relation to that on MEA was similarly judged (+, ++, and +++). Colony coloration was described.

Mycological examination of the big bale grass silage samples. A moisture chamber was prepared as follows: a sheet of 12.5-cm-diameter sterilized filter paper was soaked with 10 ml of sterile water in a 13.5-cm-diameter petri dish. Silage material from a sample was mounted in a moisture chamber and incubated for 10 days at 25°C in normal atmosphere. The moisture chamber was then examined with a stereomicroscope, and any fungi present were subcultured.

The remaining material was cut with flamed scissors and chopped in a food processor. Five grams of each sample was added to 45 ml of a 0.9% (wt/vol) aqueous solution of NaCl and kept at room temperature for approximately 30 min. This mixture was then shaken and diluted to final concentrations of 0.1, 0.01, 0.001, and 0.0001 g of sample per ml. From each dilution, 0.1 ml of inoculum was spread on the surfaces of MYSA and DRBC plates, with duplicate plates for each medium.

All plates were incubated in the dark in plastic bags for 7 days at 25°C in normal atmosphere. At the end of the incubation period, the plates were read according to the following procedure. The number of colonies was counted on all plates on which the growth of fungi was not too dense for reliable counting. The number of colonies allowing a reliable count was dependent on the floras, particularly on the quantity of fast-growing fungi, and varied to between 50 and 200. The average number of colonies on the duplicate plates was recorded. In this investigation, the number of molds refers to CFU per gram (wet weight) of sample.

From the moisture chamber and plates, representatives of each of the different colony types were subcultured and identified. Each species isolated from one sample was considered an isolate. For subcultivation, MEA, CYA, yeast extract-sucrose agar (28), creatine sucrose agar, nitrite sucrose agar (12), potato dextrose agar (4), Spezieller Nährstoffarmer Agar (22), and Sabouraud sucrose medium (2) were used. Except for the plates with creatine sucrose agar and nitrite sucrose agar, which were incubated at 20°C, all plates were incubated at 25°C for 7 days in normal atmosphere.

The *Penicillium* spp. isolated were subcultured on MEA, CYA, yeast extract-sucrose agar, creatine sucrose agar, and nitrite sucrose agar. Some isolates were inoculated in apples according to the methods of Frisvad (12). The isolates were identified according to Pitt (24), Frisvad (12, 13), and Frisvad and Filtenborg (14). The *Aspergillus* spp. isolated were subcultured on MEA and CYA. They were identified to the group level according to the method of Raper and Fenell (26). Isolates belonging to the *Aspergillus glaucus* group were subcultured on CYA with 20% sucrose in order to provoke the teleomorphic *Eurotium* state.

The *Fusarium* spp. were subcultured on potato dextrose agar and Spezieller Nährstoffarmer Agar and identified according to the method of Nelson et al. (21).

The mucorales (*Mucor* spp., *Rhizopus* spp., and *Absidia* spp.) isolated were subcultured on MEA and potato dextrose agar and identified to the genus or species level according to the methods of Schipper (29) and Samson and van Reenen-Hoekstra (28).

The *Geotrichum* spp. were subcultured on MEA and Sabouraud sucrose medium. They were identified according to the methods of Barnett et al. (2) and Samson and van Reenen-Hoekstra (28).

The yeasts were observed microscopically but were not identified.

The other fungi isolated were identified to the genus or species level according to the methods of Barnett and Hunter (1), Carmichael et al. (6), Domsch et al. (8, 9), Ellis (10, 11), and Samson and van Reenen-Hoekstra (28).

Statistical methods. The statistical method used was the chi-square test, and P values of <0.05 were considered significant.

RESULTS

Of the 571 samples examined, mold growth was demonstrated from 372 samples on MYSA and from 335 samples on DRBC. Mold growth was recorded on MYSA but not on DRBC from 86 samples and on DRBC but not on MYSA from 49 samples. In 118 samples mold growth was demonstrated only from material in the moisture chamber, while no mold growth was demonstrated from 32 samples. Molds were recorded from 7% more of the samples on MYSA than those on DRBC, and thus the recovery of molds was significantly better ($\chi^2 = 146.01$; $P < 0.001$) on MYSA. The average numbers of total CFU per gram of mold in silage samples were 9.3×10^5 on MYSA and 8.9×10^5 on DRBC. The difference in the average numbers of CFU of fungi cultivated on MYSA and on DRBC was not statistically significant. The corresponding number of yeasts was 2.6×10^6 on both MYSA and DRBC. Bacterial growth was registered from four samples of big bale

TABLE 2. Subjective evaluation of colony characteristics of fungi growing on MYSA, DRBC, and MEA

Species	No. of isolates	Colony characteristics on medium[a]:								
		MYSA			DRBC			MEA		
		Aerial mycelium production	Conidium production	Colony density	Aerial mycelium production	Conidium production	Colony density	Aerial mycelium production	Conidium production	Colony density
Alternaria alternata	5	+	+++	+++	+	++	+++	+	+++	+++
Aspergillus candidus	9	++	+++	+++	+	++	+++	+	+++	+++
Aspergillus flavus	5	++	+++	+++	++	+++	+++	++	+++	+
Aspergillus fumigatus	9	+	+++	+++	+	+++	+++	+	+++	++
Cladosporium cladosporoides	16	−	+++	+++	−	+++	+++	−	+++	+++
Eurotium herbariorum	11	+	+++	+++	++	+++	+++	−	+++	+++
Fusarium avenaceum	16	++	+	+++	+	+	++	+++	+	++
Fusarium culmorum	16	+++	+	+++	++	+	++	+++	+	++
Fusarium poae	16	+++	+	+++	++	+	+++	+++	+	+++
Microdochium nivale	10	++	++	+++	+	++	++	+	++	++
Mucor circinelloides	8	+	+++	++	++	+++	++	+	++	+
Mucor hiemalis	1	++	+++	+++	++	+++	+++	++	+++	++
Penicillium citrinum	4	+	+++	+++	−	++	+++	−	+++	++
Penicillium expansum	16	−	+++	+++	−	++	+++	−	+++	++
Penicillium fellutanum	3	−	−	+++	−	−	+++	−	+++	+++
Penicillium roquefortii I	16	+	+++	+++	−	+++	++	−	+++	++
Penicillium roquefortii II	16	+	+++	+++	−	+++	++	−	+++	++
Penicillium verrucosum	10	−	−	+++	−	−	+++	−	+++	+++
Rhizopus stolonifer	6	+	+	+	+++	+++	++	+++	+++	+++
Trichoderma viride	8	−	+	++	++	−	++	−	+	+

[a] −, not visible; +, poor or low; ++, average; +++, abundant or high.

grass silage on both MYSA and DRBC, from two samples on MYSA only, and from two samples on DRBC only.

All fungal cultures tested grew well on the media offered. Table 1 shows the minimum, maximum, and mean colony sizes measured after 7 days of incubation on MYSA, DRBC, and MEA. The mean sizes of colonies of examined cultures relative to the size of colonies on MEA were 89% on MYSA and 57% on DRBC. The radial growth of *Rhizopus stolonifer* was not reduced on either MYSA or DRBC, while that of *Mucor circinelloides* was reduced to one-third on DRBC but not reduced on MYSA. Both *Aspergillus candidus* and *Penicillium citrinum* had mean colony diameters 1.05 times larger on MYSA than on MEA. The density of the colonies was high on MYSA, and the colony margins were sharply outlined. Boundaries between colonies were distinct, and plates that were crowded, i.e., with 100 to 200 colonies, could easily be counted. Colony densities and the production of aerial mycelia and conidia, subjectively judged, are shown in Table 2. As shown, the difference between growth on MYSA and on DRBC is minor compared with the difference between growth on MEA and on these two media.

Both MYSA and DRBC suppressed growth of spreading molds. Figure 1 shows cultures of *Rhizopus stolonifer*, *Mucor circinelloides*, and *Fusarium culmorum* on MYSA, DRBC, and MEA plates.

Altogether, 63 fungal species were isolated from big bale silage samples on MYSA plates, while 57 species from the same samples were isolated on DRBC plates. The number of isolates was 22% higher from MYSA plates than from DRBC plates. Table 3 shows the numbers of isolates and species from silage material arranged in alphabetical order according to the genera isolated. The numbers of isolates of genera of important mycotoxin-producing storage fungi like *Aspergillus* spp., *Byssochlamys* spp., and *Penicillium* spp. were generally lower on DRBC than on MYSA, even if *Aspergillus versicolor* was isolated from three samples and *Aspergillus ochraceus* was isolated from one sample on DRBC only. *Penicillium roquefortii* was isolated from 190 samples on MYSA plates and from 178 samples on DRBC plates. The genera isolated only on MYSA were *Acremonium*, *Alternaria*, *Emericelliopsis*, *Ajellomyces*, *Papularia*, *Pestalotia*, *Sordaria*, *Torula*, *Ulocladium*, and *Verticillium*, while the genus isolated only on DRBC was *Pleiochaeta. Absidia corymbifera*, *Aspergillus niger*, *Aureobasidium pullulans*, and *Chrysonilia* sp. were isolated only from material in the moisture chamber (Table 4).

The coloration of colonies growing on DRBC was certainly influenced by the rose color of the medium. Reverse coloration ranged from intense pink to nuances of violet. The color of the DRBC medium was also reflected in the aerial mycelia, but strongly colored conidia to a great extent kept their original coloration. The surface as well as reverse coloration of colonies growing on MYSA was comparable to that of the corresponding colonies on MEA, although the MYSA colonies had more intense shades.

DISCUSSION

The results show that MYSA fulfils most of the six criteria given by Pitt (25) and thus may be considered a medium suitable for the enumeration and isolation of molds and yeasts from silage. MYSA suppresses bacterial growth, without affecting the growth of fungi common in feeds. Thus, MYSA seems to be nutritionally adequate for fungi of current interest, but the radial growth of fungal colonies is reduced compared with that on MEA, which promotes accurate counting. However, the major characteristic of MYSA that makes it the medium of choice for the enumeration of fungi from silage samples is that the developing fungi are easily recognized.

The growth of filamentous fungi or yeasts does not seem to be quantitatively affected by use of MYSA, since there were no significant differences when we compared the average numbers of CFU in silage counted on MYSA and on DRBC. In addition

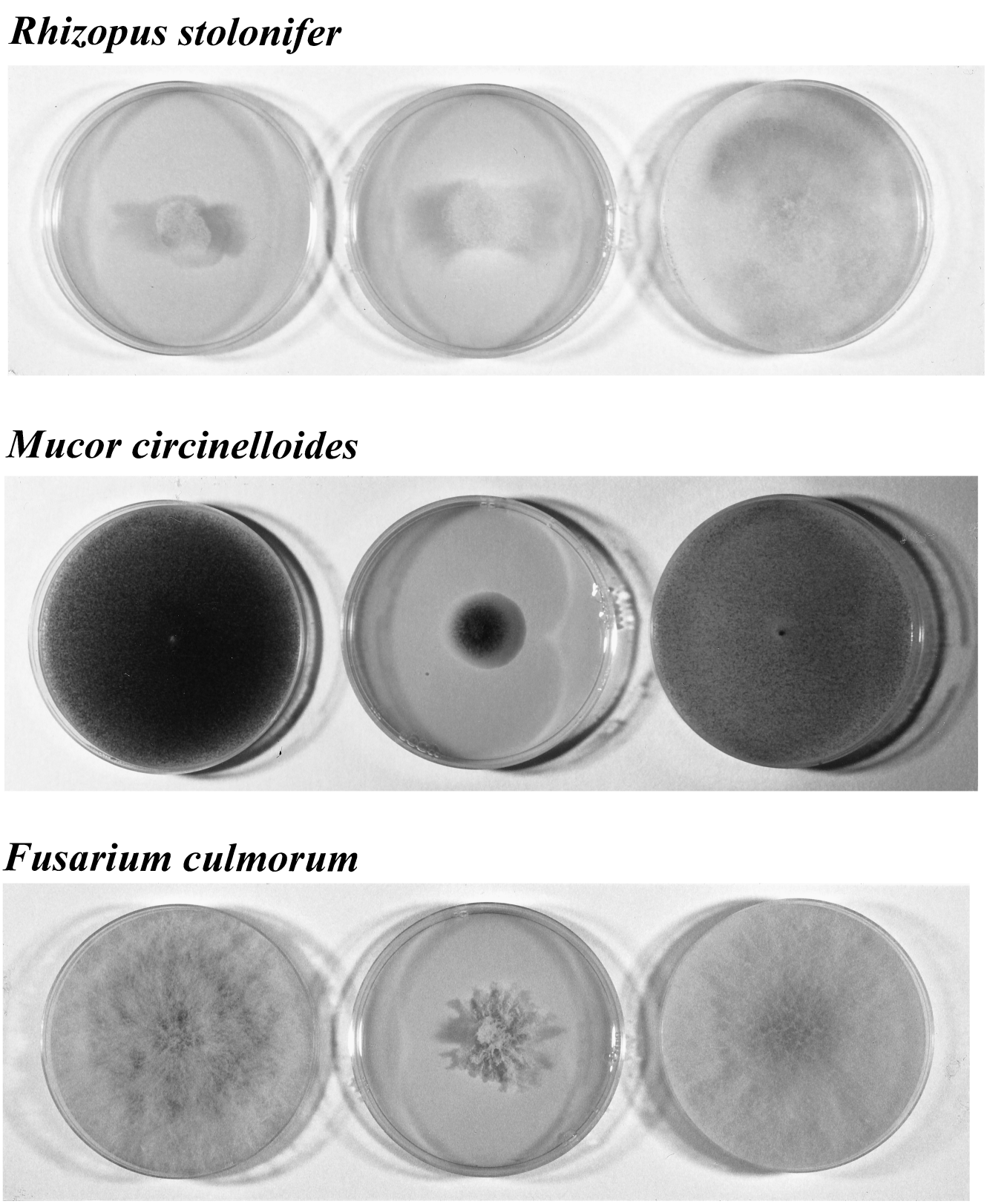

FIG. 1. The growth of *Rhizopus stolonifer*, *Mucor circinelloides*, and *Fusarium culmorum* on MYSA, DRBC, and MEA.

TABLE 3. Genera, number of species, and number of isolates from 571 samples of big bale silage on MYSA and DRBC

Genus	MYSA (sample no. = 372)		DRBC (sample no. = 335)	
	No. of species	No. of isolates	No. of species	No. of isolates
Acremonium	1	2		
Ajellomyces	1	1		
Alternaria	1	1		
Arthrinium	1	9	1	6
Aspergillus	3	118	5	101
Byssochlamys	1	7	1	4
Chaetomium	2	2	1	1
Cladosporium	1	13	1	8
Emericellopsis	1	1		
Eurotium	2	14	3	10
Fusarium	3	5	3	3
Geotrichum	2	43	2	37
Monocillium	1	2	1	1
Mucor	3	87	4	65
Paecillomyces	3	3	2	2
Papularia	1	1		
Penicillium	22	268	22	239
Pestalotia	1	1		
Phialophora	1	3	1	3
Pleiochaeta			1	2
Pseudeurotium	1	1	1	1
Rhizopus	1	21	1	12
Scedosporium	1	4	1	2
Scopulariopsis	2	8	2	7
Sordaria	1	1		
Torula	1	1		
Trichoderma	1	1	1	2
Ulocladium	1	1		
Verticillium	1	2		
Wardomyces	1	1	1	1
Other	1	1	2	2
Total	63	623	57	509

the qualitative recovery of molds was significantly better on MYSA than on DRBC. Both MYSA and DRBC suppress bacterial growth almost completely. Hocking (16) recommended that chlortetracycline, which was the antibiotic used in the original formula (18), be replaced by chloramphenicol. In the formula for DRBC used in the present work, chlortetracycline was added, not replaced. By doing so, MYSA and DRBC contained the same antibiotics, although the concentration of chloramphenicol was 25 times higher in MYSA than in DRBC and the concentration of chlortetracycline was 5 times higher in MYSA than in DRBC. Miller et al. (20) showed in a study that a 2% concentration of oxgall in the plating medium reduced the number of bacteria to 2.4 to 16% and the number of actinomycetes to 1.8 to 14%. The number of fungi was not diminished by this concentration of oxgall. However, ingredients in DRBC may affect the growth of some fungi. Recovery of *Cladosporium* spp. and nonsporulating molds have been reported to be significantly reduced by rose bengal (5). For these reasons it was recommended that a medium containing rose bengal should be used only in combination with less inhibitory media (5). Ottow (23) reported that light-exposed rose bengal inhibits actinomycetes. He suggested that fungi could be similarly affected if light degrades rose bengal to toxic compounds.

MYSA appears to be nutritionally adequate to support the growth of a relatively wide range of fungi. Its nutritional components are malt extract, yeast extract, tryptone, and sucrose in MYSA, and DRBC's nutritional component is glucose. An effect of oxgall noted by Miller et al. (20) is that the aerial mycelia are well developed in the presence of oxgall, and in this way oxgall seems to act as a nutritional source for some fungi.

The great advantage with DRBC is that the combination of dichloran and rose bengal inhibits the production of aerial mycelia and reduces colony size. This simplifies counting. The radial growth of fungal colonies is reduced on MYSA but not to the same extent as on DRBC. Miller et al. (20) found that the radial spread of fungus colonies growing on plates containing oxgall was inhibited. The fungal colonies that developed on the medium with oxgall grew with a more definite margin than on medium without oxgall (20). Although the radial growth was reduced on both MYSA and DRBC compared with that on MEA, the reduction was greater on DRBC. The colonies on MYSA were dense and had sharply outlined margins.

This study showed that MYSA has a certain inhibitory effect on fast-growing fungi, such as *Rhizopus* spp. and *Mucor* spp. A few spreading molds, such as *Absidia corymbifera* and *Chrysonilia* sp., were isolated only from material in the moisture chamber. However, since the material in the moisture chamber was undiluted, it may be that the concentrations of these fungi in the material were too low to be detected after dilution of the samples. According to Pitt (25), an enumeration medium should promote the growth of fungi relevant to contamination or spoilage and suppress the growth of irrelevant fungi. Certainly, it is difficult to select fungi of no relevance for silage quality. Storage fungi and so also the fast-growing fungi may be of particular interest.

A comparison of the macroscopic characteristics and the evaluation of the colony appearance as expressed on MYSA and DRBC with those on MEA shows that fungi growing on MYSA are easily recognized without subcultivation, in contrast to fungi grown on DRBC. This may be the chief quality of MYSA, which makes it the medium of choice for the enumeration of fungi in silage. The color of a colony is an essential characteristic for recognition of fungi. Careful examination of the colonies showed that surface colorations were comparable

TABLE 4. Genera, number of species, and number of isolates from 118 samples of big bale silage in which molds were demonstrated in a moisture chamber only

Genus	No. of species	No. of isolates
Absidia	1	2
Alternaria	1	1
Arthrinium	2	2
Aspergillus	5	165
Aureobasidium	1	1
Byssochlamys	1	22
Chrysonilia	1	1
Cladosporium	1	6
Fusarium	1	1
Geotrichum	1	15
Mucor	3	45
Paecillomyces	1	1
Penicillium	24	169
Pleiochaeta	1	1
Pseudeurotium	1	1
Rhizopus	1	93
Trichoderma	1	3
Ulocladium	1	1
Verticillium	1	2
Total	48	530

on MYSA, DRBC, and MEA. However, when the colony size was reduced as on DRBC, the colors appeared less visible. The reverse coloration could not be assessed on DRBC agar, as the spectrum of reverse colors was very limited. The fact that the colonies growing on DRBC were not readily identifiable is perhaps the major drawback of the medium. Thus, it is necessary to make subcultures, which takes time and is expensive. The differences in numbers of isolates obtained on MYSA and on DRBC may be explained by the appearance of the colonies. Although all colonies suspected to be of different species or subspecies were subcultured, some strains may have been missed because of the lack of variation in colony appearance of different taxa on DRBC. The reduced recovery of certain molds with rose bengal led to the recommendation that a medium containing rose bengal should be used only in combination with less inhibitory media, which would make each examination expensive. The present study indicates that insofar as there is a need for a supplement to MYSA, to detect fungi present in low concentrations, a simple moisture chamber can meet the demand. However, DRBC is an excellent medium if the aim is to find specific slowly growing fungi in a mixed flora.

MYSA and DRBC are both easy to prepare. MYSA is more expensive than DRBC, mainly because of higher concentrations of agar, yeast extract, oxgall, and carbohydrate. Nevertheless, the cost of the MYSA plates should be counterbalanced by the cost of plates as well as by reduced time and labor because of the increased need of subculturing prior to identification.

Since MYSA has been used as the standard medium for routine mycological examinations of animal feedstuffs at the Central Veterinary Laboratory of Norway for several years, corresponding evaluations of the medium should be pursued also for feedstuffs other than silage.

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REFERENCES

1. **Barnett, H. L., and B. B. Hunter.** 1972. Illustrated genera of imperfect fungi, 3rd ed. Burgess Publishing Company, Minneapolis, Minn.
2. **Barnett, J. A., R. W. Payne, and D. Yarrow.** 1983. Yeasts: characteristics and identification. Cambridge University Press, Cambridge.
3. **Beckers, H. J., E. de Boer, K. E. Dijkmann, B. J. Hartog, J. A. van Kooij, D. Kuik, N. Mol, A. J. Nooitgedagt, M. D. Northolt, and R. A. Samson.** 1986. Comparison of various media for the enumeration of yeasts and molds in food, p. 66–76. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
4. **Booth, C.** 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom.
5. **Burge, H. P., W. R. Solomon, and J. R. Boise.** 1977. Comparative merits of eight popular media in aerometric studies of fungi. J. Allergy Clin. Immunol. **60:**199–203.
6. **Carmichael, J. W., W. Bryce Kendrick, I. L. Conners, and L. Sigler.** 1980. Genera of hyphomycetes. University of Alberta Press, Edmonton, Alberta, Canada.
7. **Deák, T., T. Török, J. Lehoczki, O. Reichart, V. Tabajdi-Pinter, and I. Fabri.** 1986. Comparison of general purpose media for the enumeration of molds and yeasts, p. 114–119. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
8. **Domsch, K. H., W. Gams, and T.-H. Anderson.** 1980. Compendium of soil fungi, vol. 1. Academic Press, London.
9. **Domsch, K. H., W. Gams, and T.-H. Anderson.** 1980. Compendium of soil fungi, vol. 2. Academic Press, London.
10. **Ellis, M. B.** 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom.
11. **Ellis, M. B.** 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom.
12. **Frisvad, J. C.** 1981. Physiological criteria and mycotoxin production as aids in identification of common asymmetric penicillia. Appl. Environ. Microbiol. **41:**568–579.
13. **Frisvad, J. C.** 1985. Creatine sucrose agar, a differential medium for mycotoxin producing terverticillate *Penicillium* species. Lett. Appl. Microbiol. **1:**109–113.
14. **Frisvad, J. C., and O. Filtenborg.** 1983. Classification of terverticillate penicillia based on profiles of mycotoxins and other secondary metabolites. Appl. Environ. Microbiol. **46:**1301–1310.
15. **Hastings, J. W., W. Y. J. Tsai, and L. B. Bullerman.** 1986. Comparison of a general purpose mycological medium with a selective mycological medium for mold enumeration, p. 94–96. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
16. **Hocking, A. D.** 1981. Improved media for enumeration of fungi in foods. CSIRO Food Res. Quart. **41:**7–11.
17. **Kellen, L. A., T. M. Smith, C. B. Hannon, and K. E. Olson.** 1986. Comparative study of media for the detection and enumeration of molds and yeasts, p. 103–106. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
18. **King, A. D., Jr., A. D. Hocking, and J. I. Pitt.** 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. **37:**959–964.
19. **Lenovich, L. M., J. L. Walters, and D. M. Reed.** 1986. Comparison of media for the enumeration of fungi from dried foods, p. 76–83. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
20. **Miller, J. J., D. J. Peers, and R. W. Neal.** 1951. A comparison of the effects of several concentrations of oxgall in platings of soil fungi. Can. J. Bot. **29:**26–31.
21. **Nelson, P. E., T. A. Tousson, and W. F. O. Marasas.** 1983. *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park.
22. **Nirenberg, H.** 1976. Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berl.-Dahl. **169:**1–117.
23. **Ottow, J. C. G.** 1972. Rose bengal as a selective aid in the isolation of fungi and actinomycetes from natural sources. Mycologia **64:**304–315.
24. **Pitt, J. I.** 1979. The genus *Penicillium* and its teleomophic states *Eupenicillium* and *Talaromyces*. Academic Press, London.
25. **Pitt, J. I.** 1986. Properties of the ideal fungal enumeration medium, p. 63–65. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
26. **Raper, K. B., and D. I. Fenell.** 1977. The genus *Aspergillus*. Robert E. Krieger Publishing Company, San Francisco.
27. **Richard-Molard, D.** 1986. Comparison of five media for the enumeration of molds in cereals and cereal products, p. 109–111. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
28. **Samson, R. A., and E. S. van Reenen-Hoekstra.** 1988. Introduction to foodborne fungi, 3rd ed. Centraalbureau voor schimmelcultures, Baarn, The Netherlands.
29. **Schipper, M. A.** 1978. On certain species of *Mucor* with key to all accepted species. Stud. Mycol. **17:**1–52.
30. **Seiler, D. A. L.** 1986. A collaborative exercise comparing media for enumerating fungi in foods, p. 83–85. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.
31. **Stenwig, H., W. Langseth, and D. Sandli.** 1992. The occurrence of trichothecenes and moulds in barley and oats sampled in Norway. Plant Breed. Acclim. Seed Prod. **37:**43–50.
32. **Williams, A. P.** 1986. A comparison of DRBC, RBC and MEA media for the enumeration of molds and yeasts in pure culture and in foods, p. 85–89. *In* A. D. King, Jr., J. I. Pitt, L. R. Beuchat, and J. E. L. Corry (ed.), Methods for the mycological examination of food. Plenum Press, New York.